

CLAIMS:

What is claimed is:

*GKAD*

1. An isolated nucleic acid comprising a nucleotide sequence encoding a secretory signal sequence comprising the amino acid sequence MRVLVLALAVALAVGDOSNLG, or variants of said amino acid sequence that comprise conservative 5 replacements thereof that retain the biological activities of directing secretion of a fusion protein from a cell and cleavage of the secretory signal sequence from the fusion protein.

2. An isolated nucleic acid of claim 1, wherein the nucleotide sequence encoding the secretory signal sequence is att cac atc cac cag cc atg agg gtg ctt gta cta gct ctt gct gtg gct ctc gca gtg ggg gac cag tcc aac ttg ggg.

3. An isolated nucleic acid of claim 1, wherein the cell from which secretion is directed is a eukaryotic cell.

4. An isolated nucleic acid of claim 1, wherein the cell from which secretion is directed is a prokaryotic cell.

5. An isolated nucleic acid of claim 1, wherein the secretory signal sequence is cleaved between the G and D residues in the VGDQ portion thereof.

6. An isolated nucleic acid of claim 3, wherein the secretory signal sequence is cleaved between the G and D residues in the VGDQ portion thereof.

7. An isolated nucleic acid comprising a nucleotide sequence encoding a fusion protein comprising a secretory signal sequence and a reporter protein;

wherein said secretory signal sequence comprises the  
5 amino acid sequence MRVLVLALAVALAVGDQSNLG, or variants of  
said amino acid sequence that comprise conservative  
replacements thereof that retain the biological activities  
of directing secretion of the fusion protein from a cell and  
cleavage of the secretory signal sequence from the fusion  
10 protein;

wherein the reporter protein is joined to the carboxy-  
terminus of the secretory signal sequence, either directly  
or by a linking amino acid sequence.

8. An isolated nucleic acid of claim 7, wherein the nucleotide sequence encoding the secretory signal sequence is att cac atc cac cag cc atg agg gtg ctt gta cta gct ctt gct gtg gct ctc gca gtg ggg gac cag tcc aac ttg ggg.

9. The isolated nucleic acid of claim 7, wherein the reporter protein is selected from the group consisting of chloramphenicol aminotransferase, green fluorescent protein or another aequorin,  $\beta$ -amylase,  $\beta$ -lactamase, luciferase,  
5 glucuronidase, alkaline phosphatase and  $\beta$ -galactosidase.

10. The isolated nucleic acid of claim 8, wherein the reporter protein is selected from the group consisting of chloramphenicol aminotransferase, green fluorescent protein or another aequorin,  $\beta$ -amylase,  $\beta$ -lactamase, luciferase,  
5 glucuronidase, alkaline phosphatase, and  $\beta$ -galactosidase.

11. An isolated nucleic acid comprising a nucleotide sequence encoding a fusion protein comprising a secretory signal sequence and a lipopolysaccharide-binding protein;

wherein said secretory signal sequence comprises the  
5 amino acid sequence MRVLVLALAVALAVGDQSNLG, or variants of  
said amino acid sequence that comprise conservative  
replacements thereof that retain the biological activities  
of directing secretion of the fusion protein from a cell and  
cleavage of the secretory signal sequence from the fusion  
10 protein;

wherein the lipopolysaccharide-binding protein is joined to the carboxy-terminus of the secretory signal sequence either directly or by a linking amino acid sequence.

12. The isolated nucleic acid of claim 11, wherein the lipopolysaccharide-binding protein is Factor C from a horseshoe crab, or a variant thereof comprising conserved amino acid replacements or insertions or deletions that  
5 retains lipopolysaccharide-binding activity.

13. The isolated nucleic acid of claim 11, wherein the nucleotide sequence encoding the secretory signal sequence is att cac atc cac cag cc atg agg gtg ctt gta cta gct ctt gct gtg gct ctc gca gtg ggg gac cag tcc aac ttg ggg.

14. The isolated nucleic acid of claim 12, wherein the nucleotide sequence encoding the secretory signal sequence is att cac atc cac cag cc atg agg gtg ctt gta cta gct ctt gct gtg gct ctc gca gtg ggg gac cag tcc aac ttg ggg.

15. A recombinant vector comprising the isolated nucleic acid of any one of claims 1-14.

16. A recombinant host cell transformed with the vector of claim 15.

17. The recombinant host cell of claim 16, wherein said cell is selected from the group consisting of a bacterial cell, a COS cell, a CHO cell, a NIH/3T3 cell, a Schneider 2 cell, a *S. cerevisiae* cell and an EPC cell.

18. An assay for heterologous gene expression comprising:

a) culturing a cell transformed with a vector comprising the isolated nucleic acid of any one of claims 7 to 10 in a culture medium to obtain a reporter protein in the culture medium; and

b) assaying the biological activity of the reporter protein to determine the amount of the reporter protein present in the culture medium.

19. A method for obtaining systemic circulation of a desired protein in a transgenic or chimeric host organism comprising:

a) providing a recombinant vector comprising (i) a nucleotide sequence encoding a secretory signal sequence comprising the amino acid sequence MRVLVLALAVALAVGDQSNLG, or variants of said amino acid sequence that comprise conservative replacements thereof that retain the biological activities of directing secretion of a fusion protein from a eukaryotic cell and cleavage of the secretory signal

sequence from the fusion protein, and (ii) a nucleotide sequence encoding the desired protein; wherein the nucleotide sequence encoding the secretory sequence is joined in frame to the 5' end of the nucleotide sequence 15 encoding the desired protein; and

b) transforming a cell of the host organism with the recombinant vector *in vivo* to obtain a transgenic host organism that expresses the desired protein in an extracellular compartment, or transforming a eukaryotic cell 20 with the recombinant vector and implanting the transformed cell in the host organism to form a chimeric host organism that expresses the desired protein in an extracellular compartment.

20. The method of claim 19, wherein the recombinant vector provided in step a) further comprises a tissue-specific promoter operatively linked to the nucleic acid nucleic acid encoding the secretory signal sequence and the 5 desired protein to obtain tissue-specific secretion of the desired protein in step b).

21. A biosensor for detecting the presence of a compound in a sample comprising a cell that expresses a receptor that specifically binds the compound in its plasma membrane and is transformed with

5 i) a recombinant vector comprising a compound-responsive promoter that is inducible or repressible by the compound;

ii) a nucleotide sequence comprising a) a nucleotide sequence encoding a secretory signal sequence comprising the 10 amino acid sequence MRVLVLALAVALAVGDQSNLG, or variants of

said amino acid sequence that comprise conservative replacements thereof that retain the biological activities of directing secretion of a fusion protein from a cell and cleavage of the secretory signal sequence from the fusion protein, and b) a nucleotide sequence encoding a reporter protein, wherein the 5' end of the nucleotide sequence b) is joined in frame to the 3' end of the nucleotide sequence a); wherein the compound-responsive promoter is operatively-linked to the nucleotide sequence ii) to obtain compound-responsive secretion of the reporter protein.

22. A method for detecting the presence of a compound that binds to an estrogen receptor in a sample comprising:  
i) contacting the biosensor of claim 21 with the sample; and  
ii) determining the amount of the reporter protein secreted by the cell.

23. The biosensor of claim 20, wherein the compound-responsive promoter is an estrogen-responsive element wherein estrogen induces transcription from the promoter and the cell expresses a receptor that specifically binds estrogens and estrogen-mimics.

24. The method of claim 22, wherein the compound-responsive promoter is an estrogen-responsive element wherein estrogen induces transcription from the promoter and the cell expresses a receptor that specifically binds estrogens and estrogen-mimics and the increase in the amount of the reporter protein secreted after contacting the cell with the sample is measured.

25. The biosensor of claim 21 or 23, wherein the reporter gene is selected from the group consisting of chloramphenicol aminotransferase, green fluorescent protein or another aequorin,  $\beta$ -amylase,  $\beta$ -lactamase, luciferase, 5 glucuronidase, alkaline phosphatase and  $\beta$ -galactosidase.

26. An isolated nucleic acid comprising a nucleotide sequence encoding a fusion protein comprising a secretory signal sequence and a desired protein;

wherein said secretory signal sequence comprises the 5 amino acid sequence MRVLVLALAVALAVGDQSNLG, or variants of said amino acid sequence that comprise conservative replacements thereof that retain the biological activities of directing secretion of the fusion protein from a cell and cleavage of the secretory signal sequence from the fusion 10 protein;

wherein the desired protein is joined to the carboxy-terminus of the secretory signal sequence, either directly or by a linking amino acid sequence.

27. A host cell comprising a vector comprising the isolated nucleic acid of claim 26.

28. A method for producing a desired protein comprising culturing a host of claim 27 under conditions wherein the desired protein is secreted from the host cell; and

5 recovering the desired protein from the culture medium.

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C<sup>2</sup> >